Massachusetts Institute of Technology Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight

Biological Research Registration Form

Instructions:
This form is available at: http://ehs.mit.edu/site/content/biosafety-resources-and-links
Please download and save this form to your computer. When completing this document please retain the format as nearly as possible and answer questions thoroughly. Complete the appropriate sections as outlined below. **All information in this form is considered confidential.**

Please indicate all relevant biosafety levels and research descriptors that describe your research.

Biosafety Level ☒BL1 ☒BL2 ☐BL2+ ☐BL3

Research Descriptors	x	rDNA	x	Biological Agents BL1		Other		Human embryonic stem cells		Induced pluripotent stem cells
		Pathogens	x	Human Materials	x	Viral Vectors		Nanotechnology		Toxin Use

Required Information. Every Principal Investigator must complete the following:
• Section 1 (General Information)
• Section 2 (Laboratory Information)
• Section 3 (Research Description)
• Section 12 (Laboratory Safe Practices and Procedures)
• Section 13 (Certification and Signatures).

Research Specific Sections. Please complete the following sections if they are applicable to the research described here. **Please indicate either that the section has been completed or is not applicable (N/A).**		Was section completed?
Section 4	Teaching Laboratory Information	☐ Completed ☒ N/A
Section 5	Use of Recombinant and Synthetic Nucleic Acid	☒ Completed ☐ N/A
Section 6	Biological Agent Use	☒ Completed ☐ N/A
Section 7	Use of Human Source Materials	☒ Completed ☐ N/A
Section 8	Use of Human Embryonic Stem (hES) Cells or Induced Pluripotent Stem (iPS) Cells	☐ Completed ☒ N/A
Section 9	Occupational Health Assessment, Medical Surveillance and Monitoring	☐ Completed ☒ N/A
Section 10	Use of Toxins	☐ Completed ☒ N/A
Section 11	Dual Use Assessment	☐ Completed ☒ N/A

When you have completed the form, please email the completed form to your BSP contact or to BSP@mit.edu. Print and sign Section 13, the Certification and Signature page and mail this page to Biosafety Program, N52-496.

Section 1. General Information:

Current Registration number: **797**	Title of Registration: **Synthetic Biology for Clinical Applications using Fungi**	
Principal Investigator **Timothy Lu**	Departmental Affiliation/DLC: **EECS/RLE/BE/SBC**	
PI email address: **timlu@mit.edu**	PI office phone: 715-4808	PI email address: **timlu@mit.edu**
Lab Research Contact: Ky Lowenhaupt	Lab Administrative Contact:	
Lab Research Contact email address: kytsing@mit.edu	Lab Administrative Contact email address:	
Lab Research Contact phone: 4-8149	Lab Administrative Contact phone: 4-6492	

Section 2. Laboratory Information:

a. List ALL Laboratories/Facilities where research is to be conducted and the corresponding biosafety level: include cold/warm rooms, equipment rooms as appropriate. Please indicate room(s) where biosafety cabinets (BSC) are located. Please include the location(s) of the autoclave used for laboratory waste sterilization prior to disposal. (The box is expandable.)

Room Number	Biosafety Level	Check box if applicable: BSCs in room	Warm/ Cold Room	Equipment Room	Human Materials	hES/iPS cells used	Autoclave location
NE47-017B	BL1	1 BSC					Biowaste boxes
NE47-019	BL1						Biowaste boxes
NE47-209 – shared lab	BL2	1 BSC					Biowaste boxes
NE47-217 – shared tissue culture room	BL2	5 BSCs			X		Biowaste boxes
NE47-235	BL2						Biowaste boxes
NE47-265	BL2						Biowaste boxes
NE47-290C – shared cold room	BL2		Cold Room				Biowaste boxes
NE47-290D – shared autoclave/dish washing room	BL2			X			Biowaste boxes
NE47-335B	BL2						Biowaste boxes
NE47-335C	BL2						Biowaste boxes
36-797	BL2						Biowaste boxes
36-799 – autoclave/dish washing room	BL2			X			Biowaste boxes
36-781	BL2						Biowaste boxes
36-789	BL2						Biowaste boxes

b. Please list or attach a list of all laboratory personnel working on this Registration at MIT, to include faculty, technical staff, graduate students, UROPS, etc. (The box is expandable.)

Laboratory Personnel			Research Materials Used (place an X in the appropriate box)			Training Completed (Enter most recent date of training)	
Name	Kerberos	UROP (Y/N)	Uses BL1, BL2 material	Uses BL2+, BL3 material	Uses human material	General Biosafety training (260c)	Bloodborne Pathogens training
Cao, Jicong	jicong	N	X	NA	X	03/09/16	03/09/16
Chang, Cheng	Cchang1	N	X	N/A	X	04/24/15	03/29/16
Chen, Ying-Chou	Yjoechen	N	X	N/A	X	03/09/16	03/09/16
Chen, Willam	wcwchen	N	X	N/A		03/09/16	03/09/16
Citorik, Robert	Rcitorik	N	X	N/A	X	03/09/16	03/09/16

Cui, Hao	cherylc	N		N.A	X	03/09/16	03/09/16
Da Luz Areosa Cleto, Sara	scleto	N	X	N/A		03/09/16	03/09/16
Farzadfard, Fahim	Ffard	N	X	N/A		03/09/16	03/09/16
De la Fuentes Nunez, Cesar	cfuente	N	X	N/A		03/09/16	03/09/16
Higashikuni, Yasutomi	tommyh	N	X	N/A	X	03/09/16	03/09/16
Harrison, Christina	tia7	N	X	N/A	X	06//02/14	2/24/17
Jerger, Logan	ljerger	N	X	N/A		07/14/16	07/14/16
Jung, Giyoung	giyoung	N	X	N/A		03/09/16	03/09/16
Jusiak, Barbara	jusiak	N	X	N/A	X	03/09/16	03/09/16
Lemire, Sebastien	Sele	N	X	N/A		03/09/16	03/09/16
Lowenhaupt, Ky	kytsing	N	X	N/A	X	03/09/16	03/09/16
Lu, Timothy	Timlu	N	X	N/A	X	03/09/16	03/09/16
Mimee, Mark	Mmimee	N	X	N/A	X	03/09/16	03/09/16
Mueller, Isaak	imueller	N	X	N/A		03/09/16	03/09/16
Nissim, Lior	Liorni	N	X	N/A	X	03/09/16	03/09/16
Nissim, Adina	anissim	N	X	N/A	X	03/09/16	03/09/16
Park, Heechul	heechul	N	X	N/A		03/09/16	03/09/16
Park, HyunJun	hyunjun	N	X	NA		03/09/16	03/09/16
Pery, Erez	erezpery	N	X	N/A	X	03/09/16	03/09/16
Purcell, Oliver	Opurcell	N	X	N/A		03/09/16	03/09/16
Roquet, Nathaniel	Nroquet	N	X	N/A		03/09/16	03/09/16
Sothiselvam, Shanmugapriya	Priya89	N	X	N/A		03/09/16	03/09/16
Sun, Qing	sunqing	N	X	N/A		03/09/16	03/09/16
Tang, Tzu-Chieh	tctang	N	X	N/A		03/09/16	03/09/16
Tham, Eleonore	etham	N	X	N/A		03/09/16	03/09/16
Wu, Ming-Ru	mingru	N	X	N/A	X	03/09/16	03/09/16
Yehl, Kevin	kyehl	N	X	N/A		03/09/16	03/09/16

Section 3. Research Description:

Outline the overall goal(s) of the research in the space below. Give enough information to assure that the purpose of the experiments and the techniques used are clear. Please use reasonably non-technical terms. Please make clear if you will be generating any antibiotic resistant biological research agents.

The Lu Lab uses the tools of Synthetic Biology to investigate and solve problems in human health and biotechnology. This includes the development of new strategies for the synthesis and delivery of bioactive compounds such as antibiotics, bacteriophages, antibodies, and hormones. Other projects focus on developing novel biomaterials for use in manufacture and medicine. The lab is always striving to identify and develop new uses for Synthetic Biology.

1: The production and secretion of therapeutic proteins in exogenous systems including *Pichia pastoris*

1.1) Optimizing expression and secretion in *Pichia and other yeasts*

2: Treatment of human diseases using synthetic biology tool and circuits

2.1) Analysis of gene networks involved in the development of neurodegenerative diseases and aging

3: Development of molecular recorders

3.1) Developing genetic and epigenetic molecular recorders for lineage tracing and recording temporal dynamics of gene networks

4. Development of RNA-interference in Saccharomyces Cerevisiae

Detailed description of projects

1.1) *Pichia Pastoris* and other yeasts for the production and secretion of proteinaceous drugs

Pichia Pastoris is used extensively in both academic research and biotechnology production as a source of recombinant proteins. It grows to a very high cell density, and can use methanol as an inexpensive carbon source. We are optimizing this system for the production of therapeutic proteins. Some of the challenges we are tackling are increasing yield, and the co-production of drugs that work synergistically. Further, we are investigating the potential of commensal yeasts to produce these proteins *in situ* in the gut, using strategies developed in Pichia.

The goal of this work is to examine the binding specificity of a set of anti-Ebola antibodies (collectively referred to as ZMapp) that we have produced in the yeast *Pichia pastoris*. In this system, the protein of interest is secreted in the culture supernatant at very high level, which allows for a simplified purification procedure. The potency of the antibodies comes from their ability to bind to, and interfere with the function of the Ebola GP1,2 glycoprotein present on the surface of the Ebola virus. We will use assays to detect the binding of the anti-Ebola antibodies to heterologously expressed glycoprotein. The section of Ebola genome encoding the glycoprotein is non-pathogenic by itself. There is no potential for viral

production or genome replication within host organism. The glycoprotein gene will be expressed within HEK293T mammalian cells. We will use vectors pCAGGS-ZEBOV-GP, and derivatives of pDisplay, as per Qui et al (2011).

The *Pichia* production platform developed in this project is of general usefulness and is also being applied at the co-production of multi-valent drugs. human growth hormone, Granulocyte-colony stimulating factor, interferon alpha 2b, anti-PD1 antibody, anti-CLTA4 antibody, Glucagon-like peptide-1 agonist, insulin, and human serum albumin are also produced or co-produced with this system with characterization of the products by a combination of HPLC, ELISA immunodetection and western blotting.

Finally we are starting work to export the strategies developed with Pichia towards other yeasts with a particular emphasis on *S. cerevisiae* and *S. boulardii* because it is a normal inhabitant of the gut, displays elevated pH resistance and may have probiotic properties. The idea is to engineer a probiotic yeast strain like *S. Boulardii* to secrete therapeutic molecules of interest in the small intestine where they can be efficiently absorbed. The small intestine epithelium is capable of transcytosis of maternal IgG by Fc receptor-mediated endocytic events. Thus attaching the molecule/protein of interest to the Fc domain of IgG can lead them to transcytose across the epithelium of small intestine.

Goals of this project:

To engineer the probiotic yeast strain to secrete efficiently.

1. -To engineer mutations in the Fc domain of human IgG to obtain optimum binding in a pH dependent manner with the Fc receptor.
2. -To tether the protein/therapeutic molecule of interest to Fc domain and show efficient transcytosis across the small intestine epithelium.

As an alternative secretion system, we will also explore fusing the therapeutic protein to the C-terminus fragment of the enterotoxin toxin of *Clostridium perfringens*. This fragment is expected to be sufficient for transcytosis through epithelial membranes but not toxic. Once fused to gaussia luciferase, IL-10, nanobodies against TNFα, IL-6, lactoferrin, IL-17, we expect the secreted hybrid proteins to transcytose through the epithelial cell layer. Note that we will only use the C-terminus of the CPE toxin. This protein fragment, termed C-CPE, is not able to lyse cells anymore and is therefore not toxic or considered to be a toxin. We will use the same expression vectors as stated above.

The ultimate goal of this work is to investigate *S. cerevisiae* or *S. boulardii* as cheap therapeutics secreting either proteins that will only act in the gastrointestinal tract, or that will get transported through the gut epithelial cell monolayer and enters the blood stream. Eventually, biological activity will be assessed in a mouse model of gut colonization but this is not currently ongoing.

We will also engineer the above-mentioned yeasts to detect host proteins and trigger synthesis of a corrective synthetic circuit (bio-sensor). We do this by expressing two synthetic transmembrane domains consisting of nanobodies against GFP, calprotectin, TNFα, IL-6, IL-10, lactoferrin, IL-17. It is furthermore linked to yERFP, the Tobacco Etch Virus (TEV) protease, a synthetic Zinc Finger Transcription factor (A. S. Khalil, *et al.*, Cell, 2012) or a luciferase reporter protein. The reporter construct consists of a synthetic promoter fused to a reporter gene of

luciferase or yERFP. Note that both the transcription factors used and the TEV protease act orthogonal to human cells, bacteria cells and yeast cells, meaning that they will only act in our synthetic system. These constructs are expressed from a TEF promoter, and selection in yeast is done by using auxotrophic strains for uracil (URA), tryptophan (Trp), leucine (Leu) or histidine (His) or by a kanamycin cassette resulting in resistance to geneticin (G418). The plasmids will either be integrated in the genome at the auxotrophic sites or be self-replication using a 2μ origin of replication. The plasmids furthermore carry an origin of replication for growth in *E. coli* (ColE1) and an antibiotic resistance cassette for selection in *E. coli* (ampicillin/carbenicillin or kanamycin).

Our ultimate goal for this project is to be able to detect inflammation in the gastrointestinal tract of mice that has inflammatory bowel disease.

2.1) Analysis of gene networks involved in the development of neurodegenerative diseases and aging

Parkinson's disease is a neurodegenerative disease that affects over 50 million people worldwide. Its cause is not understood, but it is known that aggregates of mis-folded proteins play a role. We are working to expand out understanding of the cellular basis of Parkinson's; our results can shed light on normal aging and on other neurodegenerative diseases.

This projects aim to develop advanced screening platforms that can systematically probe and dissect the complex genetic networks in human disorders, such as Parkinson's disease and chronological aging. As a proof of concept, we used budding yeast (*Saccharomyces cerevisiae*) as a model organism to discover gRNAs that can protect cells from alpha-synuclein induced cell death. The downstream genes regulated by gRNAs were identified by next-generation sequencing approaches. We then verified the human homologs of highly ranked hits from the yeast screens in human neuroblastoma cells (SH-SY5Y) with the lentiviral system (FuGW). In these experiments gRNAs that target each of the human homologues of promising genes are introduced singly into human neuroblastoma cells, cells are challenged with alpha-synuclein. Alpha-synuclein induces apoptotic cell death in these cells; the ability of each gRNA to protect the cell will be evaluated.

With a similar approach, one of our ongoing projects is to explore the complex circuitry of signaling networks in yeast chronological aging by engineering artificial transcription factors, such as dCas9-VP64, to reprogram transcriptional networks as a repair mechanism for malfunction. This study aims to find novel expression sets which have not been identified by single-factor studies or systems-biology approaches that impact the lifespans of mammalian post-mitotic and non-dividing cells, such as neurons.

3.1) Developing genetic and epigenetic molecular recorders for lineage tracing and recording temporal dynamics of gene networks

The ability to keep a log of biological events is a valuable tool for the understanding of those events, for example during development and aging. Current methodology requires taking destructive testing at each time point. The use of molecular recorders will allow the whole log book to be read at a long time point.

We aim to develop advanced molecular recorders for recording temporal dynamics of gene networks and recording molecular events during development. We use CRISPR-Cas9 gene editing (either Cas9 nuclease, Cas9 nickase or recently described variants of Cas9 fused to cytidine deaminases) to introduce mutations in the yeast genome in a desired target locus. The mutations can be made conditional by driving the expression of guide RNA or Cas9 under control of inducible promoters. We trace the accumulation of these genetic changes in the target locus over time by high-throughput sequencing. We use *Saccharomyces cerevisiae* to demonstrate the system is functional. If successful, we plan to apply the system to mammalian cell lines (HEK) cells or alternatively be used as a way to trace cellular lineages during development.

4. Development of RNA-interference in *Saccharomyces cerevisiae*

RNA interference (RNAi) is an invaluable tool in the study and regulation of gene networks. This system exists in higher eukaryotes, but is not present in *S. cerevisiae*. We are developing an advanced version of RNAi, which will be tunable, to allow gene expression be regulated at many levels. This tool will provide a more fine grained understanding of molecular events.

We aim to develop RNAi as a tunable tool for modulating gene expression, using *S. cerevisiae* as a platform. Argonaute and Dicer from *S. castelli* are introduced into *S. cerevisiae* to endow the cell with RNAi capabilities. The development then concerns to type and structure of the targeting hairpins used with this system and the manner in which they are constructed.

Section 4. Teaching Laboratory Information: Check box if not applicable ☒

a. Please complete the bulleted points below.

- Number of students in the class:
- Student-Instructor Ratio:
- Experience level of course Teaching Assistants:
- Describe how any biological materials will be handled and who will handle the biological materials:

Section 5. Use of Recombinant or Synthetic Nucleic Acid: **Check box if not applicable ☐ (Please complete this section if you use or generate recombinant microorganisms, cells, animals, plants, etc.)**

Please indicate the Section of the NIH Recombinant DNA Guidelines that applies to this research. If you have questions Biosafety Staff will be glad to assist you. Note that the City of Cambridge requests that we register all recombinant work even if it is exempt under section III-F or Appendix C.

Section III-A ☐ **Section III-B** ☐ **Section III-C** ☐ **Section III-D** ☒ **Section III-E** ☒ **Section III-F** ☒

a. Source of Gene, Insert or Clone:

1. Specify DNA/RNA source (or probe), nature of insert, is a protein expressed, and percent of any viral genome in construct:

1. Human growth hormone (hGH) from Uniprot
2. Interferon alpha-2b from Uniprot
3. Granulocyte-colony stimulating factor (G-CSF) from Uniprot
4. Nivolumab from DrugBank
5. Ipilimumab from DrugBank
6. Pro-glargine from DrugBank
7. Human serum albumin (HSA) from Uniprot

ZMapp monoclonal antibodies; 2G4, 4G7 and 13C6. These are all chimeric antibodies with a murine variable region and a human constant region.

Dicer and Argonaute genes from Saccharomyces Castelli.

Cas9 and its variants (nCas9, dCas9) individually or fused to cytidine deaminase or TdT. Guide RNAs.

The 20 nucleotides of gRNA recognition sequences were cloned from synthesized oligos.

Alpha-synuclein was cloned from human wild-type *SCNA.*

Yeast genes were sub-cloned from Thermo Scientific Open Biosystems Yeast ORF Collection or directly PCR-amplified from yeast genome.

Systematic Name	Standard Name	Systematic Name	Standard Name	Systematic Name	Standard Name
YBL086C		YGR128C	UTP8	YPL226W	NEW1
YBR056W		YNL173C	MDG1	YBR238C	
YBR280C	SAF1	YBR147W	RTC2	YBR296C	PHO89
YDR016C	DAD1	YCR098C	GIT1	YDL018C	ERP3
YDR101C	ARX1	YDR074W	TPS2	YDR100W	TVP15
YDR106W	ARP10	YDR345C	HXT3	YEL039C	CYC7
YER058W	PET117	YGL101W		YER054C	GIP2
YGR008C	STF2	YHL021C	AIM17	YFR003C	YPI1
YHR136C	SPL2	YIL101C	XBP1	YGL120C	PRP43
YJL144W		YJL109C	UTP10	YHR126C	ANS1
YLR043C	TRX1	YKR067W	GPT2	YIL053W	GPP1
YLR119W	SRN2	YMR049C	ERB1	YLL052C	AQY2
YLR164W	SHH4	YMR290C	HAS1	YML123C	PHO84
YLR390W	ECM19	YNL305C	BXI1	YOR292C	
YMR322C	SNO4	YPL230W	USV1	YPR151C	SUE1
YNL007C	SIS1	YGR230W	BNS1	YAL028W	FRT2
YNL112W	DBP2	YPL123C	RNY1	YBR230W-A	
YOR054C	VHS3	YBR126W-A		YBR285W	

YPL280W	HSP32	YCL073C	GEX1	YBR302C	COS2
YDR358W	GGA1	YCR021C	HSP30	YDR169C-A	
YEL020W-A	TIM9	YDL110C	TMA17	YDR258C	HSP78
YDR171W	HSP42	YDR516C	EMI2	YDR342C	HXT7
YER121W		YEL012W	UBC8	YGR027W-B	
YGL258W-A		YER053C-A		YHR086W-A	
YGR247W	CPD1	YFR042W	KEG1	YHR087W	RTC3
YLR149C		YGR130C		YJR005C-A	
YNL036W	NCE103	YGR131W	FHN1	YLR401C	DUS3
YOL114C		YHR171W	ATG7	YML132W	COS3
YPL196W	OXR1	YHR197W	RIX1	YMR247W-A	
YBL039C	URA7	YJL161W	FMP33	YMR262W	
YDL199C		YJL163C		YOL161C	PAU20
YKL100C		YKL221W	MCH2	YOL164W-A	
YMR244W		YLR257W		YOR341W	RPA190
YNR002C	ATO2	YML052W	SUR7	YPR010C	RPA135
YOL084W	PHM7	YMR128W	ECM16		
YOR161C	PNS1	YNL141W	AAH1		
YPL247C		YOL032W	OPI10		
YHR075C	PPE1	YOL048C	RRT8		
YPL093W	NOG1	YOR280C	FSH3		
YFL012W		YPL012W	RRP12		

Human genes that were tested for alpha-synuclein protection were RT-PCR amplified from human neuroblastoma SH-SY5Y and then sub-cloned into FuGW (lentiviral) expression vectors.

Yeast Gene	Human Homologs
SNO4/HSP34	PARK7
HSP32	PARK7
HSP42	HSPB1, HSPB3, HSPB6, HSPB7, HSPB8, HSPB9
SIS1	DNAJB1-B9
GGA1	GGA1, GGA2, GGA3
SAF1	ALS2, RCC1
TRX1	TXN, TXNDC2, TXNDC8
TIM9	TIMM9
OXR1	OXR1, NCOA7, TLDC2

STF2	SERBP1, HABP4

2. Do any sequences code for toxins? If yes, please specify. **No**
3. Is the DNA source from a USDA-regulated plant, animal or insect? If the regulated organism is grown or stored at MIT, please include a copy of the USDA permit. (Link to USDA site: http://www.aphis.usda.gov/brs/index.html) **No**

b. Vectors and Host Cells:

1. Identify cloning/expression/transfection vectors used, recipient bacterial strains, and recipient host cell lines (human, mouse, plant, etc.). Provide a restriction map of vector if it is new or not published. If commercially available vector or plasmid, please indicate vendor. Describe the location and type of promoters and other control sequences and percent of any viral genome in construct.

I. Cloning in yeasts

Saccharomyces cerevisiae.

S. cerevisiae strains used will typically be W303, S288c, EG123, YPH500 and their derivatives. Work in *S. cerevisiae* uses Fluorescent reporters (GFP, RFP etc) and their variants (e.g. yeast-enhanced GFP). The original sources of fluorescent reporters differ, but the original source of GFP was from the jellyfish *Aequorea victoria*. The RNA-interference system from the related yeast *S. castelli* has also been introduced into *S. cerevisiae*. Hairpin structures used in conjunction with the RNAi system are all synthesized synthetically and are not directly derived from any known sequence or organism. These genes are cloned in plasmids of the pRS4xx family (https://www.neb.com/~/media/NebUs/Page%20Images/Tools%20and%20Resources/Interactive%20Tools/DNA%20Sequences%20and%20Maps/pRS413_map.pdf). pRS40x plasmids are integrative (no yeast replication origin) and differ by their selective marker (HIS, LEU, TRP or URA). The pRS41x collection possesses a yeast replication origin based on a min centromere (low copy) whereas the pRS42x family have a yeast 2μ origin that allows replication at high copy number. (Selection markers used are the auxotrophic markers (URA, HIS, LEU, TRP etc), G418 or Zeocin. cas9 and dcas9 may be used for editing of DNA and transcriptional regulation respectively and are integrated into the genome of yeast after cloning into pRS404 or 405. Neither of these proteins by themselves confers any level of pathogenicity.

Saccharomyces boulardii.

We adapted the MoClo-system (a cloning strategy; Weber et al., 2011) for constructing integrative/replicative plasmids in *Saccharomyces boulardii*. The resulting method was used to screen for combinations of constitutive promoters, secretion leaders, genes (GLuc, IL-10, IL-22, TGF-b, Fc-domain, C-terminus of *clostridium perfrigens* toxin [termed CCPE – the C-terminus by itself is not toxic]), selection markers (URA, HIS, LEU, TRP, G418, Zeocin) and then integration/2μ to test secretion in *boulardii* of these proteins. All standard fluorescent reporters (GFP, RFP etc) may also be used. cas9 and dcas9 may be used for editing of DNA and transcriptional regulation respectively. Neither of these proteins or combinations are expected to confer any level of pathogenicity.

Pichia Pastoris

We will use vectors pCAGGS-ZEBOV-GP, and derivatives of pDisplay, as per Qui et al (2011). *P. pastoris* strains used will include the wild-type, GS115 and Glysoswitch. Projects are interested in the production of therapeutic biologics, such as interferon and monoclonal antibodies.

Selection markers used: kanamycin/G418, Zeocin
Genes inserted:
1) Fluorescent proteins: GFP, RFP and CFP.
2) Therapeutic proteins: human growth hormone (hGH), interferon alpha-2b, Granulocyte-colony stimulating factor (G-CSF), Nivolumab, Ipilimumab, ZMAPP, pro-glargine, human serum albumin (HSA), IL-10, IL-22, TGF-b, CCPE.
3) Other proteins: zinc-finger protein, lac repressor protein, *S. cerevisiae* alpha-mating factor secretion signal, tobacco etch virus (TEV) protease, GLuc, Fc-domain, *Aspergillus aculeatus* β-glucosidase (BGL1), *Trichoderma reeseii* endoglucanase II (EGII).
None of these proteins confers any level of pathogenicity.

II. Cloning in cell lines

SH-SY5Y (ATCC® CRL-2266™)

2. If using viral vectors, indicate packaging cell lines and assay system used to measure helper virus titre or titre of replication competent virus (background) generated. Include host range of packaged viral vector. (If using retroviral or lentiviral vectors additional requirements apply. Please see MIT CAB/ESCRO policy at the following URL: http://web.mit.edu/cab/policies.html

	Lentivirus
description	HIV-based lentiviral system, pLV CAG CN-2A-CN (AAVS1 Zinc finger)
Source	A platform for rapid prototyping of synthetic gene networks in mammalian cells. Duportet et al., 2014
Viral vector	3rd generation lentiviral vector and its derivatives (E.g., pFUGW vector and etc.)
Packaging cell lines	HEK-293T (ATCC® CRL-3216™), SH-SYSY
Assays for measuring helper virus and detecting replicative competent virus	To test for the presence of replication-competent or helper virus, marker rescue experiments will be performed. Culture supernatants of 293T cells which have been infected with a replication-defective virus expressing the puromycin resistance gene will be used to infect a second set of 293 cells. The second set of 293 cells will be selected in puromycin for several weeks to identify infected cells. This is a sensitive technique since a single colony, arising from a single replication-competent virus, can be detected readily or In order to test for the presence of replication competent or helper virus, we perform standard RT-PCR or real time PCR to determine the presence of lentiviral-related RNA transcripts. In addition, we test for horizontal transfer from supernatant of infected cells; supernatant of 293T cells, infected with replication-defective virus expressing GFP, is harvested and used to infect a second set of 293T cells. Test cells are prepared for FACS analysis at various time points for several weeks to determine the presence of GFP+ cells, an indication of infection by replication competent virus. This is a sensitive technique since a single GFP+ cell, arising from a single replication-competent virus, can be detected readily. These tests will be performed for each construct. The properties of particular plasmids should not change over time since they are stable at -20°. (1)
Replicative incompetent	Yes
Pseudotype	VSV-G
Host range	Mammals
Safety feature	SIN, 3-plasmid system
Integrate into cells	Do not infect efficiently in vivo; The vectors used are self-inactivating vectors
Risk of exposure (i.e. needlesticks, sharps injury, contact with non-intact skin, or splash	Potential for oncogenesis

and splatter into eyes or mucous membranes of nose and mouth)	
Risk if researcher receives an exposure, i.e. insertional mutagenesis,	Insertional mutagenesis, HIV virus may occur in HIV+ individuals or by in vitro recombination resulting in replication competent lentivirus.

1. Dull, T., R. Zufferey, M. Kelly, R. J. Mandel, M. Nguyen, D. Trono, and L. Naldini. 1998. A third-generation lentivirus vector with a conditional packaging system. *J Virol* 72:8463-8471.

c. Use of Animals, Plants or Insects: Check box if not applicable ☒
Contact the MIT Committee on Animal Care (IACUC) to register your animal research. The questions below are intended to deal with the use of potentially biohazardous agents in animals or the creation of transgenic animals, plants or insects.

1. If transgenic, including "knockout", animals/plants/insects will be generated, please provide information on the transgene and vector as well as the expected phenotype of the recombinant animal/plant/insect.
   None to be generated.

2. Does this recombinant species pose a risk to the community or the environment if there is an inadvertent release outside the laboratory or animal facility? n.a.

d. Large-Scale Research: Check box if not applicable ☒

Do experiments involve growth of 10 liters or more of culture at a time? If YES, identify culture room and type of equipment used for large-scale culture growth and handling.

Section 6. Biological Agent Use: Check box if not applicable ☐

(Please complete this section if you work with viable microorganisms or viruses.)

1. Please complete this section for all viable microorganisms used in this research project including viruses, bacteria, archea, fungi, protozoa, or algae.

   Multiple strains of the same species may be entered on the same row if they are known or hypothesized to exhibit similar virulence in their host(s). Please list the name(s) of all strains in the appropriate column.

   Disinfectants used in the lab: 10% (v/v) household bleach; 70% ethanol; Quaternary ammonia compound; and iodophor. For spore formers and those strains that 70% ethanol are not effective, 10% (v/v) household bleach will be used

Genus and species	Strain(s)	Risk Group (click link for assistance determining RG) †	Biosafety Level	Source (e.g. name of collaborator or company)
Escherichia coli	DH5alpha, S17 lambda pir,	1	1 (Lab is BL2)	CGSC, Michael Fischbach (UCSF), CGSC, T. K. Lu

	MG1655, Nissle 1949			
Saccharmyces cerevisiae	W303, S288c, EG123, YPH500, BY4741, YVH10	1	1	Maheshri Lab, GE lifesciences, Collins lab, ATCC
Pichia Pastoris	GS-115, Glycoswitch, ATCC 76273 , X-33	1	1	ATCC, Thermo Fisher, biogrammatics, Love lab
Saccharomyces boulardii.	Sb48, M2, SJ50, SJ52-55, SJ79, SJ80	1	1	ATCC, Lamb lab, Jin lab

2. Please complete the following section for all human pathogens (i.e. Risk Group 2 or 3 organisms) listed above as well as any known opportunistic pathogens. **Please copy the table for each organism**.
3. Are any of the organisms listed in part 1. above known to cause disease exclusively in any animal or plant (i.e. not a human pathogen)? Choose an item. NO

Section 7. Use of Human Source Material: Check box if not applicable ☐

a. Do you have an Exposure Control Plan (ECP) on file with the MIT EHS office? YES_X_ NO___

b. If no, then how has the material been treated prior to use in the lab (such as formalin fixing or heat treatment)? Please describe how material will be rendered noninfectious prior to use.

Section 8. Human Embryonic Stem Cells or Induced Pluripotent Stem Cells (hES or iPS Cells respectively): Check box if not applicable ☒

Investigators should be aware of the NIH Guidelines on Human Stem Cell Research if they plan to work with either cell type (http://stemcells.nih.gov/policy/2009guidelines.htm).

1. **Do you plan to derive human embryonic stem (hES) cells?**
   Yes ___ No____ If yes, please describe the technology e.g. single cell nuclear transplantation, derivation from a donated embryo, etc. Please contact the Biosafety Office at 2-3477 as we will need further information. Please note that NIH will not fund derivation of hES cell lines.

2. **Are the human embryonic stem cells (hES) with which you plan to work on the NIH Registry of federally approved lines?** Yes___ No____ If yes, please list cell line(s) and indicate where you will get the hES cell line.

3. **If you plan to use an already existing human stem cell line that is not on the NIH Registry please provide the following information as an attachment to this Biological Research Registration:**
   a. Please list hES Cell Lines and source: another laboratory or investigator
   b. Documentation required as part of registration (from the source investigator and institution)
      i. Please submit a Letter of Assurance from the investigator supplying the cells. This letter should document that the hES cell lines were generated with Institutional Review Board (IRB) oversight and approval. Please be sure that the source investigator includes the name of the approving IRB and the IRB OHRP assurance number.
      ii. Please submit the approval letter from the Institutional ESCRO Committee.
   c. MIT investigators must document that a source of non-federal funding for research involving these particular hES cell lines is in place.

d. A plan must be developed to ensure separation of supporting materials and equipment for work with all non-federally approved hES cell lines.
e. Contact the Office of Sponsored Programs for information on funding issues surrounding research involving non-federally approved hES cell lines.

4. **Funding Information: Please list all grants and contracts, including pending grants or contracts, that describe the use of any hES cell based research. Please include funding source e.g., NIH, HHMI, JDRF, etc., and start and end dates. If departmental or MIT funds are used please indicate that as well (enter "internal MIT funds" into grant/contract space).** (The box is expandable.)

Funding Agency	Grant/ Contract Number	Internal Cost Object	Date Grant Submitted	Date Grant begins	Date Grant ends

Does the information in this biological research registration form include all of the laboratory research section(s) described in the grants or contacts listed above?

☐Yes ☐ NA (internal MIT funds to support this research only) ☐ No (Explain)

5. **Do you plan on isolating and developing human induced pluripotent stem (iPS) cells or cell lines?** Yes____ No______ If yes, please describe the source of the somatic cells to be used and complete Section 7 of this form. In addition you will need to develop an Exposure Control Plan. Is the donor traceable? Yes____ No_____

6. **What methods will be used to generate the induce pluripotent stem cells?** If you use viral vectors to generate the iPS cells please complete the detailed questions in Section 5 of this form.

7. **Do you plan to inject or transplant hES or iPS cells in any animal, animal embryo or at any stage or prenatal development where the hES or iPS cells may contribute to the animal germline?** Please outline the animal research in the space below.

Section 9. OCCUPATIONAL HEALTH ASSESSMENT, MEDICAL SURVEILLANCE & MONITORING: Check box if not applicable ☒

Some research may involve the need for a health assessment or vaccination prior to the initiation of the project. If there is a health risk associated with this research, please check the appropriate box below and contact Occupational Medicine. You can contact Occupational Medicine at 617-253-8552 to arrange an appointment. Occupational Medicine assessment is available to all MIT employees/investigators regardless of the biosafety level of the research

- ❑ **Pre-project serum samples.** These samples of blood serum are collected prior to beginning work with some types of infectious materials to serve as a reference should an infection occur during the course of work with an agent.

- ❑ **Pre-project vaccinations other than the Hepatitis B vaccine.** A vaccination may be warranted based on the nature of the work being done and the availability of an appropriate vaccine. Check the box if you need a vaccine other than Hepatitis B. Type of Vaccine:_______________

- ❑ **Medical Surveillance monitoring**. This may include a baseline assessment, periodic evaluations during the experiment time period, and a final evaluation at the end of the experiment. **Note:** This type of surveillance is not usually indicated for research below Biosafety Level 3.

Section 10. Use of Toxins:

Check box if not applicable ☒

Please contact the BSP at 2-3477 to discuss special ordering, storage procedures and standard operation procedures.

HHS & USDA Select Agent Toxins	Max. qty. in lab (mg)	Working dilution	Use in Animals? Y/N	Lockbox/ logbook location
Abrin				
Conotoxins				
Diacetoxyscirpenol (DAS)				
Ricin				
Saxitoxin				
Tetrodotoxin				
Shiga-like ribosome inactivating proteins				
Botulinum neurotoxins				
Clostridium perfringens epsilon toxin				
Shigatoxin				
Staphylococcal enterotoxins				
T-2 toxin				
Other Toxins of Biological Origins				
Diphtheria toxin				
Vibrio Cholera, Subunit A				
OTHER:				

1. Please describe the overall use of toxins of biological origins including Select Agent Toxins.
2. Attach your toxin standard operation procedure (SOP). The toxin SOP should include health & safety risks, security information, safe handling procedures, personal protective equipment, disposal/inactivation procedures for contaminated equipment, sharps, solid waste, etc. A template is available on EHS website: https://ehs.mit.edu/site/sites/default/files/files/Select-Agent-Toxin-SOP-template(1).doc

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Section 11. Dual-Use Assessment:

☐ Yes ☒ No. If yes, please indicate which question number and answer below.

1. Will the experiment(s) result in acquisition of new characteristics such enhanced virulence, infectivity, stability, transmissibility, or the ability to be disseminated? If so, explain:
2. Will the experiment(s) result in resistance to useful prophylactic or therapeutic interventions? If so, explain:
3. Will the experiment(s) result in the biological agent being able to evade detection methodologies as such that the capacity to identify or provide treatment for the agent? If so, explain:
4. Will the experiment(s) enhance the susceptibility of a host to the biological agent? If so, explain.
5. Will the experiment(s) cause disruption in the immunity of the host or the effective next of an immunization or change the host range? If so, explain:
6. Will the experiment(s) generate or reconstitute a biological agent for which there are no known or widely available prophylactic or therapeutic interventions? If so, explain.
7. Will your research result in the development of materials or technologies with “dual use” potential? If so please explain:

Section 12. Safe Practices and Procedures: Please complete this section for all viable biological research agents or materials excluding human-derived materials. If your biological research involves only the use of human-derived materials, check this box ☐ and proceed to the next section.

1. Please identify and discuss the health and safety risks associated with the proposed research use of this biological agent or recombinant materials.

For viral vectors, please see section 5.

BL1 bacterial strains (ex: DH5alpha, One Shot TOP10, NEB 5-alpha) used in the lab are non-infectious to humans. The same is true with all yeast strains (Saccharomyces or Pichia). They are all safely used following BL1 practices and procedures.

Human cell lines are covered in the Exposure Control Plan (please see document for more detail) and follow BL2 practices and procedures

2. Describe the signs and symptoms of infection, the mode of transmission, availability of vaccine or therapeutic treatment. Is it zoonotic, i.e. animal to humans, humans to animals, or humans to humans? **See Section 6.**

3. What procedures create the greatest risk of exposure or infection e.g. aerosolization of materials, and how will this risk be minimized during the course of the research: **No research involves processes highly likely to aerosolize materials, eg. Waring Blender. Work with potentially infections materials will be performed in a biological safety cabinet or in a sealed anaerobic chamber;**

4. Outline protective equipment required to minimize exposure of laboratory personnel during all procedures requiring handling or manipulation of biological agent e.g. use of gloves, lab coats, safety glasses, etc.

All researchers working in the lab are required to wear gloves and lab coats as well as closed toed shoes and long trousers/skirts. Safety glasses are provided and researchers are instructed on which activities require use of safety glasses. Biosafety cabinets are used for the manipulation of mammalian cells, all work with viral vectors, and work with pathogens.

5. Outline decontamination procedures and disinfectant(s) to be used for work surfaces, instruments, equipment, liquid containing biological materials and glassware:

   Work surfaces, instruments, and equipment are decontaminated with Sklar spray and wipes. Liquid containing materials are dispensed into bleach to a final concentration of 10% (v/v) bleach, wait 20 minutes and pour down the sink. Glassware is treated with 10% (v/v) final concentration of bleach, rinsed thoroughly, washed then autoclaved.

6. Outline disposal/decontamination procedures for contaminated sharps, contaminated solid waste, tissues, pipette tips, etc. **All biologically contaminated solid waste is disposed of through the biological waste disposal system of EHS biowaste boxes. Items contaminated with pathogens will be treated with 10% (v/v) final concentration bleach before disposal in biowaste box or for liquids, wait 20 minutes and pour down the drain. Sharps are contained in a designated box before being put in the biowaste box. Surfaces, including the floor, are cleaned with commercial disinfectant or 10% (v/v) final concentration bleach.**

7. Will mixed waste be generated (radioactive/biological or chemical/biological)? YES NO **X**
   If yes, please indicate how you will inactivate the biological component of the mixed waste in the

box below. (For information on waste management, please see: http://ehs.mit.edu/site/waste. If you have questions, contact EHS at 2-3477).

Section 13. Certification and Signatures

The information contained in this application is accurate and complete. I am familiar with and agree to abide by all guidelines and regulations pertaining to this research. These guidelines and regulations include the current NIH Guidelines for Research Involving Recombinant DNA Molecules; CDC and NIH guidance documents such as “Biosafety in Microbiological and Biomedical Laboratories”; the DHHS and USDA Select Agents and Toxin regulations; OSHA Bloodborne Pathogen Standard; the provisions of the City of Cambridge Ordinance on Recombinant DNA Research; the Massachusetts State Sanitary Code Chapter VIII, 105 CMR 480, “Minimum Requirements for the Management of Medical or Biological Waste; Massachusetts law, M.G.L. c.111L, “Biotechnology” for human embryonic stem cell research; as well as any MIT Policies and Procedures and other local, state and federal regulations that may be applicable.

Specifically I agree to abide by the following requirements:

a. I will not initiate any biological research subject to the regulations and guidelines mentioned above until that research has been registered, reviewed and approved by the Committee on Assessment of Biohazards and Embryonic Stem Cell Research Oversight (CAB/ESCRO). The purview of the MIT CAB/ESCRO includes biological research involving recombinant DNA; biological agents and pathogens; human cells, tissues, materials and embryonic stem cells; select agents and toxins, and the use of any of the above in animal research.
b. I will assure that personnel have received appropriate information about the biological hazards of the research outlined in this registration by making available copies of approved protocols, Biosafety Manuals, and Biological Research Registrations that describe the potential biohazards and precautions to be taken to prevent exposures or release to the laboratory or the environment.
c. I am familiar with and will ensure use of appropriate biosafety level laboratory practices and procedures in the conduct of this research.
d. I certify that laboratory personnel have appropriate technical expertise.
e. I will ensure that laboratory personnel know the procedures for dealing with incidents and spills of biological materials, and know the appropriate waste management procedures.
f. I will comply with all shipping requirements for biohazardous materials.
g. I will ensure that all laboratory personnel working with biological materials are listed on this registration.
h. I will assure that all laboratory personnel have completed all necessary training and that their training records are up to date.
i. I certify that all laboratory spaces associated with the research described in this registration are listed.
j. If this research involves recombinant or synthetic nucleic acid technologies, I am familiar with and understand my responsibilities as a Principal Investigator as outlined in Section IV-B-7 of the “NIH Guidelines for Research Involving Recombinant DNA Molecules” (a copy of this section is available from the MIT Biosafety Program)
k. I will assure adequate supervision of personnel, and will correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research as listed above.
l. I will inform the MIT Biosafety Program of any serious spills, potential exposures or breaches of the guidelines and regulations listed above.

20 January 2016

Principal Investigator — Date

MIT Biosafety Officer Date